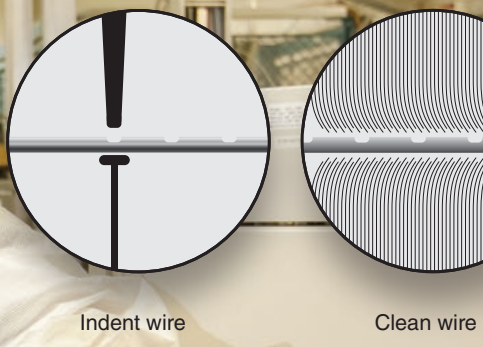


Faster Analysis from Biological Accelerator Mass Spectrometry

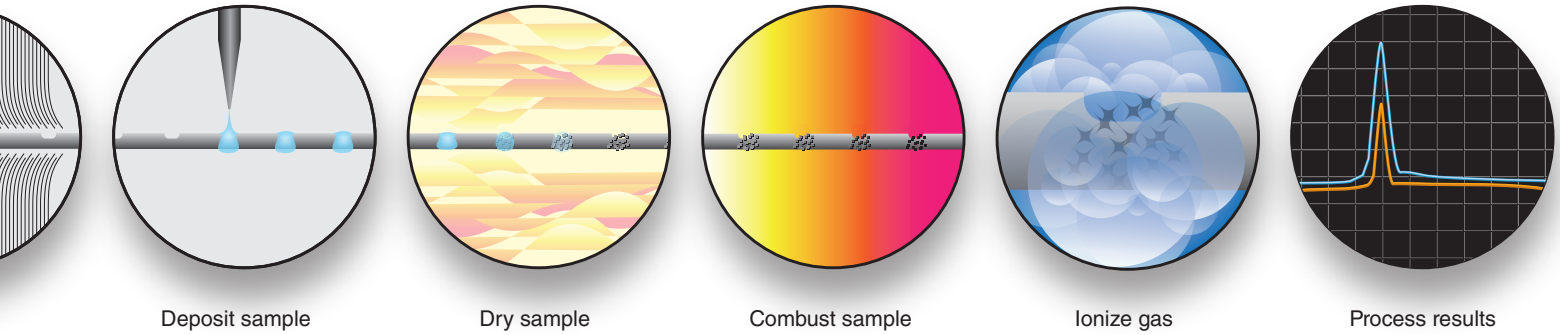
Avi Thomas works with a biological accelerator mass spectrometry (bioAMS) instrument at Livermore. The system includes a high-performance liquid chromatograph and a moving-wire interface, which allow researchers to directly study liquid samples.

THE search for a new drug—whether to treat cancer or a flu pandemic—is time consuming and costly, requiring up to 15 years and hundreds of millions of dollars to turn an idea into an effective product. Tom Baillie, the dean of pharmacy at the University of Washington, adds that "Just one of the 10,000 molecules studied early on by chemists will make the grade. There are lots of expensive failures along the way."

High-throughput screening techniques and combinatorial chemistry play an important role in the initial screening process for new medications. Another approach that holds great promise for drug discovery is accelerator mass spectrometry (AMS), an extremely accurate method for dating bones, tree rings, ice cores, and other carbon-bearing materials.

AMS uses an accelerator to count the carbon-14 atoms in a sample. It is sensitive enough to find one carbon-14 isotope among a quadrillion other carbon atoms. With that number, researchers can quickly and accurately deduce a sample's age because the carbon-14 level in the sample will mirror the isotope's level in the atmosphere at the time the material was created.

In the late 1980s, Livermore scientists were the first to apply the sensitivity of AMS to biological testing. In biological AMS (bioAMS), a substance to be studied is tagged with carbon-14 or another radioactive isotope and ingested or absorbed by a test subject. In the hours, days, or weeks that follow, the carbon-14 isotope will show up in the subject's DNA, blood, urine, or tissue. Over the last 25 years, bioAMS research has revealed how animals



BioAMS relies on a moving-wire interface to transport liquids to the accelerator. A tiny wire is indented at regular intervals and then cleaned. When liquid droplets from the high-performance liquid chromatograph land on the wire, they are attracted to the indentations. Solvent in the sample is then removed through evaporation, and the remaining material is reduced to carbon dioxide and ionized. This gas is transferred to the accelerator, which measures the ratio of carbon-14 to either carbon-13 or carbon-12 in the material.

and humans metabolize carcinogens, vitamins, and toxins. (See the box on p. 20.)

AMS is unique in that it can measure extremely low concentrations of substances with a high level of accuracy. To study how humans respond to an existing or candidate medication, Lawrence Livermore researchers and medical collaborators developed a technique called microdosing, in which a patient takes just 1/100 of a normal therapeutic dose. The drug's "fate" in the patient's body can easily be measured and studied.

Baillie, who served as vice president of drug metabolism and pharmacokinetics at Merck Research Laboratories before moving to the University of Washington, looks forward to having AMS readily available for human trials required in the drug-discovery process. He estimates that researchers could shave at least a few months off the development timetable by using the technique.

He is not alone in his enthusiasm. "The pharmaceutical and medical research communities want to apply bioAMS in a routine, nonresearch fashion," says Ken Turteltaub, who leads the Laboratory's bioAMS program. "But the instrumentation must be a lot simpler and easier for nonexperts to use."

Faster, Cheaper, and Easier

The traditional AMS technology requires that all sample material be reduced to solid graphite pellets. This step involves physicists and experts in sample preparation, precise chemistries, and days of work—all factors that make bioAMS expensive and severely limit its utility in a nonresearch setting.

Livermore scientist Ted Ognibene is working with Avi Thomas, a Lawrence scholar and physics doctoral student at the University of California (UC) at Davis, to develop a sample-preparation method that accommodates liquid samples and bypasses the graphitization process. Their technique rapidly converts the carbon content of liquid samples to carbon dioxide and transports the gas to an ion source, where it is ionized before it enters the accelerator. With the new process, samples can be much smaller—nanograms instead of micrograms—and AMS results are available in minutes. Thomas's dissertation explains the process in detail.

A commercial high-performance liquid chromatography (HPLC) unit allows for the use of liquid samples. This separation and analysis tool is found in most biology and medical laboratories. In fact, when athletes are tested for substance abuse, HPLC units process their urine, separating each sample into individual components for analysis.

The innovation that transports liquid to an accelerator is a moving-wire interface. Moving-wire interfaces have been adapted for some applications but have not previously been used with AMS. A tiny wire, indented at regular intervals, accepts the HPLC output—a nonvolatile sample material dissolved in liquid. The surface area of the precisely made indentations attracts the liquid, ensuring that samples are exactly the same size. The wire proceeds to a drying oven to evaporate the solvent in the sample. It then moves to a combustion oven, where the sample's carbon content is converted to carbon dioxide gas. (See the movie at str.llnl.gov/Dec12/images/bioams.wmv.)

AMS quantifies the carbon isotopes by separating the ions derived from a sample and identifying their nuclear charge and mass. The amount of carbon-14 is measured relative to the more abundant carbon-13 or carbon-12 isotopes in the ion beam. These separation and measurement steps follow the traditional AMS process. What has changed dramatically is the time required. With standard AMS, researchers typically had to wait days or sometimes weeks to obtain test results. In the new bioAMS process, from the moment a sample is deposited on the wire until its carbon ratio is measured, a mere 90 seconds have elapsed.

In addition, traditional AMS often required the entire sample for a single measurement. Says Thomas, "With our changes, scientists will likely have material left over after the bioAMS analysis. If the experiment needs to be tweaked or repeated for some reason, they will have something left to examine. And with the fast turnaround, the experiment can be run again almost immediately."

Biological Accelerator Mass Spectrometry at Livermore

In 1988, Livermore scientists used accelerator mass spectrometry (AMS) to determine how low doses of a suspected carcinogen affect the DNA of mice. AMS achieved a tenfold improvement in detecting damaged DNA over the best methods then available.

In the years since, researchers at the Center for Accelerator Mass Spectrometry have expanded AMS for biomedical and pharmaceutical applications. The center has become the world leader in biological AMS research, and in 1998, the National Institutes of Health (NIH) named it the first NIH Research Resource for Biological AMS. Researchers from Livermore and institutions worldwide have applied AMS to answer questions in fundamental biology, metabolism, nutrition, toxicology, pharmacology, and more recently drug development.

The traditional AMS processes used to perform these studies have been relatively expensive and time consuming. The current work at Livermore is facilitating the use of smaller samples and reducing the time required for sample preparation and processing. These steps will save money and offer fast results to researchers, moving biological AMS one step closer to routine laboratory use.

AMS in a Biology Lab

In 2014, a bioAMS system funded by the National Institutes of Health will be installed at Livermore. The spectrometer will still be large, occupying a small room. However, with the new HPLC and moving-wire interface components, it will be a unique and powerful analytical tool for biomedical research. According to Graham Bench, the director of Livermore's Center for Accelerator Mass Spectrometry, physicists will help get the new system running properly, and the center's team will be on call for troubleshooting the initial experiments. The goal, however, is for biomedical researchers to use the instrument as they would any other piece of laboratory equipment, which will make the bioAMS unit different from other AMS systems.

Ralph deVere White, an M.D. and director of the UC Davis Comprehensive Cancer Center, has been a bioAMS booster for years. "Not only can AMS and microdosing be used to discover new drugs, but we are also using them right now for clinical trials with an existing chemotherapy drug for bladder cancer," says deVere White, a long-time collaborator with Turteltaub and others at Livermore. "Patients respond differently to medications, and we are trying to predict who will respond to the chemotherapy treatment and who won't. Thanks to bioAMS, we may be able to make better use of a drug we already have."

—Katie Walter

Key Words: biological accelerator mass spectrometry (bioAMS), Center for Accelerator Mass Spectrometry, drug discovery, high-performance liquid chromatography (HPLC), moving-wire interface, pharmaceutical research.

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